chondria were used immediately after isolation. All data are based on from two to five independent determinations.

The data reveal that the uncoupling efficiency of a given concentration of DNP depends upon the substrate metabolized. At 5×10^{-5} *M*, DNP uncoupled approximately 50% of the phosphorylation associated with α-ketoglutarate oxidation. This same concentration of DNP had relatively little effect on the phosphorylation coupled with the oxidation of α-glycerophosphate, succinate and glutamate. At a higher level of DNP, 1×10^{-4} *M*, phosphorylation associated with α-ketoglutarate oxidation was almost completely eliminated. In contrast, phosphorylation coupled with glutamate oxidation was only slightly reduced. With the respiratory substrates, succinate and α-glycerophosphate, moderate decreases in the phosphate esterified were observed. Neither concentration of DNP caused marked alterations in the respiratory rate. Other experiments with a further five-fold increase in DNP, 5×10^{-4} *M*, completely uncoupled phosphorylation with all substrates.

These data indicate that phosphorylations coupled to electron transfer are not equally sensitive to DNP. This implies that during oxidative phosphorylation steady state concentrations of the components of the system are established and that this balance regulates the response to a given concentration of DNP. In our experiments the different substrates may have modified this equilibrium by influencing the steady state levels of respiratory enzymes[9] or by having different phosphorylation sites.[10] Other evidence demonstrates that the oxidation of these substrates in the fly is mediated by the same respiratory chain components as found in mammalian preparations.[11,12] In both organisms the initial electron acceptor for α-ketoglutarate and glutamate is pyridine nucleotide. This coenzyme is not a participant in the mitochondrial oxidation of α-glycerophosphate.[13] Thus it is unlikely that the apparent contrast in our results with some previously reported data with mammalian tissues[14] can be attributed to gross differences in the electron transport system. This comparison therefore suggests that the differing effects of a given concentration of inhibitor may be a reflection of differing equilibrium states.

(9) B. Chance and G. Williams, *J. Biol. Chem.*, **217**, 409 (1955).
(10) B. Chance and G. Williams, *ibid.*, **217**, 439 (1955).
(11) B. Sacktor, *J. Gen. Physiol.*, **35**, 397 (1952).
(12) B. Sacktor, *Arch. Biochem. Biophys.*, **45**, 349 (1953).
(13) B. Chance and B. Sacktor, unpublished results.
(14) F. Hunter, in "Phosphorus Metabolism," (W. D. McElroy and B. Glass, editors), The Johns Hopkins Press, Baltimore, Md., 1, 297 (1951).

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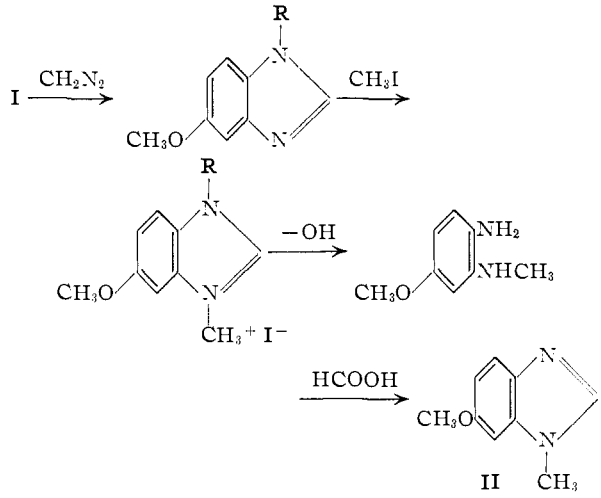
VITAMIN B_{12}. XXVII. STRUCTURE OF THE FACTOR III NUCLEOSIDE AND SYNTHESIS OF HYDROXY- AND METHOXYBENZIMIDAZOLE RIBOSIDES

Sir:

It has been reported previously that Factor III contains a 5- or 6-hydroxybenzimidazole moiety[1,2] and appears to differ from vitamin B_{12} only by having this moiety in place of 5,6-dimethylbenzimidazole.[3] The following evidence shows that the nucleoside of Factor III is a 5-hydroxybenzimidazole glycoside, probably an α-ribofuranoside.[4] Factor III was kindly supplied by Professor Dr. K. Bernhauer.

The nucleoside (I)[1] obtained by acid hydrolysis of Factor III was not isolated in crystalline form but was purified by paper chromatography using an *n*-butanol–acetic acid–water (4:1:5) system (R_f 0.46). The absorption spectrum in methanol showed λ_{max} at 249 and 296 mμ, and the $[\alpha]^{25}D$ was about −11°.

Since 5-hydroxybenzimidazole could result from degradation of either a 5- or 6-hydroxybenzimidazole glycoside the position of the hydroxyl group in I was shown as follows



The Factor III nucleoside I was methylated with diazomethane and the product was converted to the methiodide by refluxing in methanol with methyl iodide. The methiodide was cleaved to an *o*-phenylenediamine by treatment with hot methanolic sodium hydroxide, conditions known to cleave N,N′-dialkylbenzimidazolium salts to N,N′-dialkyl-*o*-phenylenediamines.[5] In the case of I, these conditions would also be expected to cause removal of the sugar moiety, giving a monomethyldiamine in which the unsubstituted amino group is derived from the original glycosidic nitrogen. For characterization, the diamine from I was converted into 1-methyl-6-methoxybenzimidazole (II), m.p. 67–68°, by reaction with formic acid. II was synthesized by first reacting 3-bromo-4-nitroanisole and methylamine to give N-methyl-6-nitro-*m*-anisidine. This was hydrogenated to the

(1) F. M. Robinson, I. M. Miller, J. F. McPherson and K. Folkers, THIS JOURNAL, **77**, 5192 (1955).
(2) W. Friedrich and K. Bernhauser, *Angew. Chem.*, **67**, 619 (1955).
(3) W. Friedrich and K. Bernhauser, *Z. Naturforschung*, **9b**, 686 (1954).
(4) By private communication, we have been informed by Professor Dr. K. Bernhauer that he and Dr. Friedrich have methylated Factor III directly, and then by degradation have obtained 6-hydroxy-1-methylbenzimidazole. Thus, they have established that the nucleoside of Factor III is a 5-hydroxybenzimidazole glycoside (W. Friedrich and K. Bernhauer, *Angew. Chem.*, in press).
(5) K. Hoffman, "Imidazole and Derivatives," Part I, Interscience Publishers, Inc., New York, N. Y., 1953, p. 280.

corresponding diamine which reacted with formic acid to give II (calcd. for $C_9H_{10}N_2O$: C, 66.65; H, 6.22; N, 17.28. Found: C, 66.89; H, 5.99; N, 17.40). A mixed melting point of the isolated and synthetic compounds was not depressed. Therefore, I was a 5-hydroxybenzimidazole glycoside.

The available knowledge made it seem likely that the Factor III nucleoside, like α-ribazole,[6] is an α-D-ribofuranoside. For comparison, two methoxybenzimidazole ribofuranosides were synthesized. Reaction of the chloromercuri derivative of 5-methoxybenzimidazole (which probably was a mixture of 1-chloromercuri-5- and -6-methoxybenzimidazoles) with triacetyl-α-D-ribofuranosyl chloride followed by deacetylation gave two products which were purified by countercurrent distribution in a butanol–water system and separated as their picrates (III) m.p. 144–146° and (IV) 78–82° (calcd. for $C_{19}H_{19}N_5O_{12}$: C, 44.80; H, 3.76; N, 13.75. Found: (III) C, 44.74; H, 3.40; N, 13.88; (IV) C, 44.36; H, 3.94; N, 13.58). Regeneration of the bases by use of IR-45 resin gave from II a crystalline base (V), m.p. 162–164°, $[\alpha]^{22}D$ −33°, and from III a non-crystalline base (VI), $[\alpha]^{21}D$ −36°. The nearly equal $[\alpha]D$ values indicate the same glycosidic configuration and therefore a difference in the position of the methoxy group. The positions of these groups were determined by the method described above. From V was obtained 1-methyl-6-methoxybenzimidazole and from VI, 1-methyl-5-methoxybenzimidazole,[7] m.p. 110–113°. Therefore V was a 5-methoxybenzimidazole riboside and VI was the 6-methoxy isomer.

Demethylation of V with 48% hydrobromic acid gave a non-crystalline product (VII) which was purified by paper chromatography (R_f 0.47), $[\alpha]^{25}D$ about −59°. The ultraviolet absorption spectra of VII and I were nearly identical. If the assumption that I is a D-ribofuranoside is correct, it is the α-form and the synthetic compound VII is the β-form.

The similarities in the behavior of the natural hydroxy- and methoxybenzimidazole glycosides with that of the corresponding synthetic ribosides lend support to the postulate that I is an α-D-ribofuranoside. Studies are now in progress to obtain further evidence concerning structure.

(6) N. G. Brink and K. Folkers, THIS JOURNAL, **74**, 2856 (1952).
(7) A. M. Simonov and P. A. Uglov, *J. Gen. Chem.*, **21**, 884 (1951).

CONTRIBUTION FROM THE MERCK, SHARP & DOHME RESEARCH LABORATORIES DIVISION OF MERCK & CO., INC. RAHWAY, NEW JERSEY
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RECEIVED JUNE 5, 1956

THE STRUCTURE OF ELAIOMYCIN, A TUBERCULOSTATIC ANTIBIOTIC

Sir:

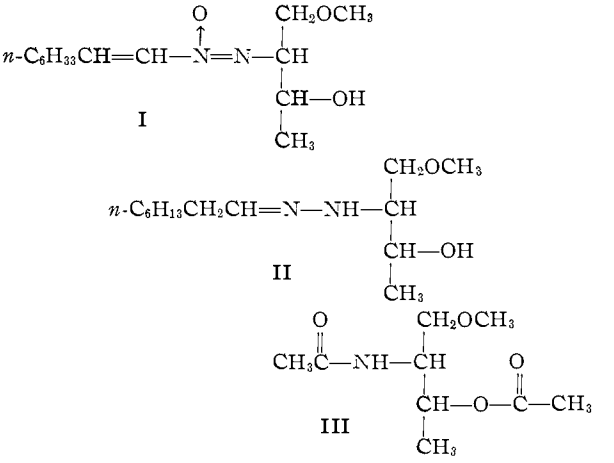
Structure I is proposed for the antibiotic Elaiomycin.[1] This antibiotic is chemically unique, since to our knowledge the chromophore represented by the *aliphatic α,β-unsaturated azoxy* group has not previously been reported.[2]

The previous characterization indicated I as an optically active, neutral oil, $C_{13}H_{26}N_2O_3$, with one alkoxyl and two terminal methyl groups. The hydrogen content allowed two double bonds and the ultraviolet spectrum (λ_{max} 237.5, ϵ = 11,000) showed them in conjugation.

The infrared spectrum of I indicated an –OH and/or possibly an –NH. The infrared spectrum of the monoacetate derivative, b.p. 84–90° (0.5 μ); λ_{max} 237.5, ϵ = 11,000; $[\alpha]^{27}D$ +25.3°, 3% in ethanol (calcd. for $C_{15}H_{28}N_2O_4$: C, 59.97; H, 9.39. Found: C, 60.28; H, 9.70), which had no absorption in the 3 μ region, indicated an acetate ester and thus confirmed the presence of the only acetylatable hydrogen as an –OH.

I and the acetate of I absorbed two moles of hydrogen (Pt, alcohol) to give II,[3] b.p. 70–80° (4 μ); λ_{max} 229, ϵ = 5300; $[\alpha]^{25}D$ +3.7°, 5% in chloroform (calcd. for $C_{13}H_{28}N_2O_2$: C, 63.89; H, 11.55; N, 11.46. Found: C, 63.31; H, 11.44; N, 10.80), and the acetate of II, b.p. 63–67° (0.5 μ); λ_{max} 230, ϵ = 4700 (calcd. for $C_{15}H_{30}N_2O_3$: C, 62.46; H, 10.48; N, 9.71. Found: C, 62.40; H, 10.60; N, 9.53).

The facile reductive loss of a single oxygen atom indicated an N-oxide, which, with the alcohol and alkoxyl groups, accounted for the three oxygen atoms. II and the acetate of II were easily hydrolyzed to *n*-octanol, providing the skeleton of the C_8 moiety.



The fact that in the acetic acid I absorbed four moles of hydrogen (Pt) or II absorbed two moles of hydrogen with the formation of two amines showed that the two fragments were joined in the original via the two nitrogen atoms and clearly indicated an azoxy group. The amines were identified as *n*-octylamine and a C_5 amine, isolated as the completely acetylated acetate-amide III, b.p. 85–90°

(1) T. H. Haskell, Q. Bartz, *et al.* [*Antibiotics and Chemotherapy*, **4**, 141, 338 (1954)] described the isolation, purification, spectra, chemical characterization, and biologic studies of Elaiomycin [4-methoxy-3-(1-octenyl-*NON*-azoxy)-2-butanol].

(2) Recently the plant poison, Macrozamin, has been shown by Lythgoe [*J. Chem. Soc.*, 2311 (1951)] to contain an asymmetrical aliphatic azoxy group. Hydroscopin A [*J. Antibiotics (Japan)*, **7**, 329 (1954)] may also be similar although no structural work has been reported.

(3) II was formulated as an aliphatic hydrazone since the ultraviolet and infrared spectra differed from those of an azo compound but resembled those of a model compound C_6H_{13}—C(CH_3)=N—NH—CH_3 (λ_{max} 229, ϵ = 5300).